* NOTICES *

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2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the outstanding antimicrobial agent with the safety high in a detail which makes a natural organic compound an active principle further about an antimicrobial agent.
[0002]
[Description of the Prior Art] Conventionally, it originates in the pericarp of a natural product, especially citruses, and it excels in antimicrobial activity, and an antibacterial substance with high safety is isolated and refined, and the example which succeeded in structure determination is not known.
[0003] On the other hand, about the psoralen (psoralen, i.e., [3 and 2-Flo g] coumarin, and alias name 7H-[3 and 2-Flo g] [1] benzopyran-7-ON) which is the compound which has a coumarin ossification center, and its derivative having antibacterial [which was excellent to the microorganism (for example the mold which are parasitic on a cavity bacillus, a gum disease Hara bacillus, or vegetation)], the research report of others [patent] from the first is not made, either.
[0004]
[Problem(s) to be Solved by the Invention] Now, although the chemical composition is unavoidably used as an antimicrobial agent, there is a problem in respect of the safety to the body or an environment. Then, it looks forward to development of the outstanding antimicrobial agent with high safety in this industry.
[0005]
[Means for Solving the Problem] This invention was made in order to respond to the needs of the above-mentioned industry, and it paid its attention to the natural product from the field of safety serious consideration.
[0006] Then, when this invention persons inquired wholeheartedly in quest of the antibacterial substance of the natural product origin, they checked that an antibacterial substance was contained in the pericarp of citruses, such as lemon and a grapefruit. And it succeeds also in those structure determination about three purification matter (LE-I, LE-II, LE-III), checks antibacterial [which was further excellent in these purification matter], and it not only succeeded in separating and refining an antibacterial substance, but results in completion of this invention at last.
[0007] That is, this invention relates to the antimicrobial agent which makes an active principle LE-I, LE-II, and/or LE-III. These LE matter is the quality of a natural product of the pericarp origin of citruses, it was a coumarin derivative, and as a result of structure determination, 8-JIERANOKISHI psoralen (8-geranoxypsoralen) and LE-II were identified 5-JIERANOKISHI psoralen (5-geranoxypsoralen), and LE-III was identified the 5-JIERANOKISHI-7-methoxy coumarin (5-geranoxy-7-methoxycoumarin) for LE-I.
[0008] These LE matter shows the antibacterial action which stood high, and is very useful as an antimicrobial agent. Hereafter, the example is described about the manufacture approach of LE matter, structure determination, and antibacterial.

[0009]
[Example 1] It extracted by leaving in a methanol the pericarp of the lemon strained lees ground by the mixer for 37 degrees C and three days. The active substance was adsorbed by resin, when this was condensed by the evaporator, the concentrate was diluted with water and the silica gel chromatography was presented. The impurity was eluted with the methanol 60%, next this was eluted with the methanol 80%, and the sample containing an active substance was obtained. Fractionation was further carried out by preparative isolation HPLC, and three fractions which show activity were obtained. By adding water gradually in the methanol solution of these three fractions, solubility was lowered and it crystallized by repeating heating and cooling. This crystallization (recrystallization) was repeated and three purification matter (LE-I, II, III) was obtained.
[0010] About these LE matter, it is each. 1H NMR and 13C NMR analysis was performed and the result of the following table 1, Table 2, and Table 3 was obtained.
[0011]
[Table 1]
LE－I：8－geranoxypsoralen

	^{1}H NMR(a)	^{13}C NMR(b)
2		160.49
3	6.37(1H, d, J=9.5)	114.68
4	7.76(1H, d, J=9.5)	144.31
5	7.36(1H, s)	113.18
6		125.80
7		148.74
8		143.12
9		143.93
10		116.45
11	5.03(2H, d, J=7.0)	70.06
12	5.60(1H, dt, J=7.0 & 1.0)	119.41
13		131.55
14	1.69(3H, bs)	16.51
15	2.01(2H, m)	39.54
16	2.01(2H, m)	26.32
17	4.99(1H, m)	123.74
18		131.69
19	1.56(3H, s)	17.62
20	1.64(3H, s)	25.62
2′	7.69(1H, d, J=2.5)	146.58
3′	6.81(1H, d, J=2.5)	106.70

(a) $CHCl_3$ as 7.26ppm ($CDCl_3$, 270MHz)
(b) $^{13}CHCl_3$ as 77.0ppm ($CDCl_3$, 67.5MHz)

[0012]
[Table 2]

LE－II：5－geranoxypsoralen

	^{1}H NMR(a)	^{13}C NMR(b)
2		161.29
3	6.28(1H, d, J=9.5)	112.52
4	8.17(1H, dd, J=9.5 & 0.5)	139.59
5		148.95
6		114.17
7		158.10
8	7.16(1H, bs)	94.19
9		152.63
10		107.48
11	4.95(2H, d, J=7.0)	69.73
12	5.54(1H, tq, J=7.0 & 1.0)	118.84
13		143.02
14	1.69(3H, d, J=1.0)	16.65
15	2.10(2H, m)	39.47
16	2.10(2H, m)	26.19
17	5.07(1H, m)	123.47
18		130.00
19	1.60(3H, s)	17.68
20	1.68(3H, s)	25.65
2′	7.60(1H, d, J=2.5)	144.85
3′	6.96(1H, dd, J=2.5 & 1.0)	105.05

(a) $CHCl_3$ as 7.26ppm ($CDCl_3$, 270MHz)

(b) $^{13}CHCl_3$ as 77.0ppm ($CDCl_3$, 67.5MHz)

[0013]
[Table 3]

LE－Ⅲ：5－geranoxy－7－methoxycoumarin

	^{1}H NMR(a)	^{13}C NMR(b)
2		161.63
3	6.15(1H, d, J=9.5)	110.76
4	8.01(1H, dd, J=9.5 & 0.5)	139.04
5		156.25
6	6.29(1H, d, J=2.0)	95.78
7		163.59
8	6.41(1H, dd, J=2.0 & 0.5)	92.67
9		156.82
10		104.25
11	4.60(2H, d, J=6.5)	65.69
12	5.48(1H, tq, J=6.5 & 1.0)	118.47
13		142.12
14	1.75(3H, bs)	16.71
15	2.12(2H, m)	39.47
16	2.12(2H, m)	26.20
17	5.09(1H, m)	123.56
18		131.96
19	1.61(3H, s)	17.70
20	1.68(3H, s)	25.63
OMe	3.85(3H, s)	55.74

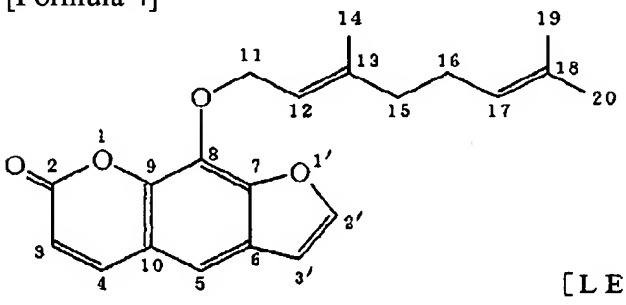
(a) $CHCl_3$ as 7.26ppm ($CDCl_3$, 270MHz)

(b) $^{13}CHCl_3$ as 77.0ppm ($CDCl_3$, 67.5MHz)

[0014] From these results, structure determination of LE matter was performed, LE-I was identified 8-geranoxypsoralen (a chemical structure type shown in ** 4), and LE-II was identified 5-geranoxypsoralen (a chemical structure type shown in ** 5), and LE-III was identified 5-geranoxy-7-methoxycoumarin (a chemical structure type is shown in ** 6).

[0015]

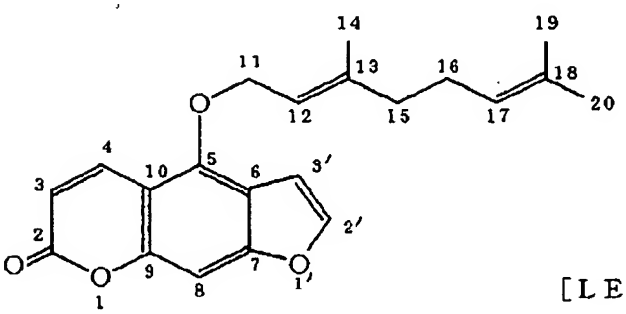
[Formula 4]



[LE－I]

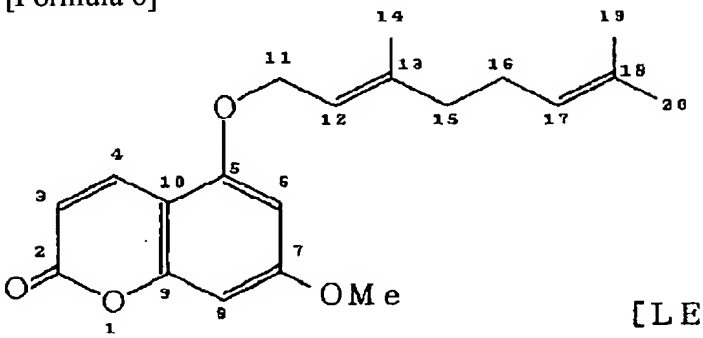
[0016]

[Formula 5]



[LE-II]

[0017]
[Formula 6]



[LE-III]

[0018]
[Example 2] The antimicrobial activity to the cavity and gum disease bacillus of LE matter was measured as follows, and was checked.
[0019] It is Streptococcus mutans known as a cause bacillus about a cavity. ATCC 7270 was used. The cause bacillus of gingivitis and periodontoclasia was used about gum disease. That is, as a cause bacillus of gingivitis, they are Prevotellaintermedia and Actinomyces viscosus. NIAH 1010 is used and it is Porphyromonasgingivalis as a cause bacillus of periodontoclasia. 381 was used. And the gum (GAM) culture medium was used as a growth medium of P.intermedia and P.gingivalis, using a brain heart infusion (BHI) culture medium as a growth medium of S.mutans and A.viscosus.
[0020] Measurement of the growth inhibition activity of LE matter to these funguses inoculated the bacillus into 5ml of bouillon culture media which added these three kinds of samples 105 cells(es)/ml, at 37 degrees C, was cultivated for two days and performed. However, about P.gingivalis, since bacteria was an anaerobe obligative, it cultivated within the aversion jar (product made from BBL). Then, the existence of a colony was observed, after adding 0.1ml of culture medium to the agar medium and cultivating this for 37 degrees C and two days.
[0021] When he had no colony, it judged antibacterial to be sterilization. And correlation of sample concentration and antibacterial effectiveness was investigated and the minimum inhibition concentration (Media Interface Connector) which sterilizes this bacillus by the minimum concentration showed the strength of activity. Moreover, LE-I, and II and III were melted to the methanol, and it checked that the methanol of an addition did not have effect in growth of a bacillus. A result is shown in the following table 4.
[0022]
[Table 4]

虫歯・歯周病原菌に対する抗菌活性、最小阻害濃度（MIC）について

	S. mutans	P. intermedia	A. viscosus *	P. gingivalis
LE－I	50ppm	50ppm	＞100ppm	100ppm
LE－II	50ppm	50ppm	＞100ppm	75ppm
LE－III	25ppm	50ppm	＞100ppm	25ppm

* A. viscosusについては、LE－I，II，IIIともに100ppmで滅菌は見られ無なかったが、接種時より菌の減少が見られ、同菌の増殖効果（制菌効果）が認められた。

[0023]
[Example 3] The growth inhibition activity over the mold generated at the time of fruits storage of LE matter was measured as follows, and was checked.
[0024] Trial mold was separated from the storage lemon fruits which mold generated. And the spore in which Penicillium digitatum of a green mold disease and Penicillium italicum of a blue mold disease were intermingled was obtained. The potato dextrose agar was used for the growth medium.
[0025] Measurement of the growth inhibition activity of LE matter to these mold created 5ml of agar media which added these three kinds of samples on the petri dish, and made the hole of 2mm of diameters centering on the petri dish. There, the 105 spores/ml mold spore was inoculated equivalent [every] (5microl) there, and was cultivated for 25 degrees C and three - seven days. The circle diameter of the hypha of the mold which grew was measured and extent of growth was observed. LE-I, and II and III were melted to the methanol, and it checked that the methanol of an addition did not have effect in growth of mold. A result is shown in the following table 5.
[0026]
[Table 5]

	3日	5日	7日
無　添　加	＋	＋＋	＋＋＋
0.05％LE－I	－	＋	＋＋
0.05％LE－II	－	＋	＋＋
0.05％LE－III	－	＋	＋＋

－　径５ミリ未満
＋　径５ミリ以上～１５ミリ未満
＋＋　径１６ミリ以上～２５ミリ未満
＋＋＋　径２５ミリ以上

[0027]
[Effect of the Invention] The antimicrobial agent of this invention was extracted from the pericarp of citruses, and its safety is very high, and it uses and is effective in mildewproofing of garden stuff. Moreover, since a cavity bacillus and gum disease bacteria reproduction inhibition activity are also high, it can add suitably for dentifrice, the detergent in opening, various food, etc., for example.
[0028] Moreover, since the structure determination of an antimicrobial agent was made by this invention, the invention of a still newer antimicrobial agent is also expectable by manufacturing the derivative of these compounds.

[Translation done.]